Historic, Archive Document

Do not assume content reflects current
scientific knowledge, policies, or practices.

A 389.9
R312
Cop. 2

_

PROGRESS REPORT

OF THE

HUMAN NUTRITION RESEARCH DIVISION

MARKETING AND NUTRITION RESEARCH

July 1, 1971

U.S. DEPT. OF AGRICULTURE
NATIONAL AGRICULTURAL LIBRARY
RECEIVED
FEB 15 1972
PROCUREMENT SECTION
CURRENT SERIAL RECORDS

Agricultural Research Service
UNITED STATES DEPARTMENT OF AGRICULTURE

Human Nutrition Laboratory Inaugurated in Grand Forks, North Dakota.

The Human Nutrition Laboratory in Grand Forks, North Dakota, a new field facility of ARS, was inaugurated by an International Symposium on the Newer Trace Elements in Nutrition. The symposium was held from September 15-17, 1970, and was attended by approximately 500 people, including scientists from Canada, Australia, and Czechoslovakia. The meeting served to discuss the latest research discoveries in trace element nutrition, among them the essential roles for three new trace elements. It also acquainted the scientific community with the Human Nutrition Laboratory of ARS, its facilities for human metabolism studies, and its important mission in human nutrition research. A book on the symposium, "Newer Trace Elements in Nutrition", is in press.

Photo on cover: Human Nutrition Laboratory,
Grand Forks, North Dakota

Issued January 1972

This progress report includes a summary of the current research of the Division and a preliminary report of progress made during the preceding year. It is primarily a tool for use of scientists and administrators in program coordination, development, and evaluation.

The summaries of progress of research include some tentative results that have not been tested sufficiently to justify general release. Such findings, when adequately confirmed, will be released promptly through established channels. Because of this, the report is not intended for publication and should not be referred to in literature citations. Copies are distributed only to those having a special interest in the development of public agricultural research programs.

The report was compiled in the Human Nutrition Research Division, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, Maryland.

This publication reports research involving pesticides. It does not contain recommendations for their use, nor does it imply that the uses discussed here have been registered. All uses of pesticides must be registered by appropriate State and/or Federal agencies before they can be recommended.

CAUTION: Pesticides can be injurious to humans, domestic animals, desirable plants, and fish or other wildlife -- if they are not handled or applied properly. Use all pesticides selectively and carefully. Follow recommended practices for the disposal of surplus pesticides and pesticide containers.

TABLE OF CONTENTS

	Page
Introduction	1
Selected Examples of Progress	2
Human Requirements for Fats and Foods to Meet These Needs	5
Human Requirements for Minerals and Foods to Meet These Needs	12
Human Requirements for Vitamins and Foods to Meet These Needs	20
Human Requirements for Protein and Amino Acids and Foods to Meet These Needs	25
Human Requirements for Carbohydrates and Foods to Meet These Needs	34
Effect of Pesticide Use on Human Dietary Requirements	41

Trade names are used in this publication solely for the purpose of providing specific information. Mention of a trade name does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture or an endorsement by the Department over other products not mentioned.

PROGRESS REPORT OF THE
HUMAN NUTRITION RESEARCH DIVISION
MARKETING AND NUTRITION RESEARCH
July 1, 1971

INTRODUCTION

The first Federal research program in human nutrition was begun by the Department of Agriculture in 1894 under the direction of Dr. W. O. Atwater. The Human Nutrition Research Division is now the major unit for human nutrition research in the Department of Agriculture, although other divisions in the Agricultural Research Service also conduct some studies in food and nutrition. The Division headquarters and most of the laboratory facilities are at the Agricultural Research Center in Beltsville, Maryland. A laboratory for metabolic studies, primarily concerned with mineral nutrition, has recently been established on the campus of the University of North Dakota in Grand Forks.

Basic information on human nutrition is needed for conservation and optimum use of food resources to promote the nutritional well-being, health, and personal satisfactions of all people in the Nation. The chief focus of the research program is to obtain information on the human requirements for nutrients and the ability of various foods to provide these nutrients. The goal is to provide a sound basis for dietary recommendations for normal individuals throughout their lives.

Division scientists cooperate with scientists of colleges and universities, State agricultural experiment stations and research institutes and associations, as well as with research workers in other units of the Department and in other Government agencies. Some of the cooperation is on an informal basis and some is formalized with cooperative agreements and memorandums of understanding. The Division's program includes domestic contract and grant research, and research in foreign countries conducted under a special foreign currency research grant program authorized by Public Law 480.

The research reported here presents recent progress in understanding the nutritional needs of normal man and the manner by which these needs can best be met by food. Studies of metabolic processes and nutritional requirements in man are preceded, guided, and expedited by results from research on laboratory animals and lower forms of life in which more factors can be controlled and physiological responses can be measured during each stage in the life cycle and in successive generations. The program includes research on the nutritive values of foods measured by chemical or physical means and by biologic response. The effects of institutional and household practices of storage, preparation and service of foods upon their nutritive value are studied. Investigations are also made of the effect of pesticide use upon human nutrition and the nutritive value of foods.

Wheat Contains A Micronutrient Essential for Efficient Utilization of Carbohydrate. Researchers of the Human Nutrition Research Division are working on the isolation and identification of a chromium-containing micronutrient, the glucose tolerance factor. This factor is required for optimal utilization of blood sugar in the experimental animal and probably also in man. Although brewers' yeast has been routinely used as starting material for the purification of the glucose tolerance factor, it has been found that whole wheat is also an excellent source. The consumption of whole wheat therefore will not only furnish energy but at the same time an essential micronutrient which is required to metabolize the carbohydrate consumed.

Vitamin C Increases the Availability of Iron in the Diet. Iron nutrition presents a major problem in the U. S. population, not because the dietary intake of this element is insufficient but because some of the iron is poorly available for absorption. In a study of iron as it occurs in different foods, ARS scientists discovered that the iron contained in eggs is only 1/5 as available as a standard iron salt. The addition of low levels of vitamin C to the diet of rats greatly increases the absorption of iron from eggs, but it does not alter the availability of iron compounds which are well absorbed anyway. Similar findings have been obtained by others in humans where orange juice appears to increase the absorption of various iron compounds. These findings, if applied in planning menus and food combinations, could greatly alleviate the existing iron deficiency problem in the U. S.

Some Negro Children Have Trouble Digesting Milk. In a recent study in selected Baltimore schools, scientists working under an ARS grant found that one fifth of the Negro children failed to consume moderate amounts of milk, as compared to one tenth of the white children. Some of these children were randomly selected for further study. Over half of the Negro children tested, milk drinkers as well as those who rejected milk, could not adequately digest lactose, the sugar in milk. Three fourths of the Negro children in this subsample who rejected milk had trouble digesting milk sugar. Only one fifth of all the white children studied, including both milk drinkers and those who reject milk, had difficulty digesting the milk sugar. Milk is an important food in child feeding programs because it is the best food source of calcium, and also contributes significant amount of other nutrients.

Even though some of these children with lactose intolerance may continue to consume milk, they are probably not realizing its full nutritional value. These facts point up the need for considerations of ways to improve the tolerance of some children for milk.

Insulin Influences Conversion of Dietary Carbohydrate to Fat. ARS scientists have found that insulin status early in life may be important in governing the metabolic pattern of the individual at maturity. Insulin is a hormone which regulates the use of blood sugar. Rats of a strain which tend to have fatty livers have an abnormally high serum insulin level in early life. These animals also have a high level of enzymes which speed up key steps in fat synthesis in the liver. Serum insulin decreases in these animals by 100 days of age to levels normal for other rats, but the enzyme pattern of the liver persists. Young rats of a second strain, which usually have low levels of the liver enzymes for fat synthesis and low levels of serum insulin, were given either insulin or a drug which stimulates the release of the rat's insulin into the blood. The treatments had no lasting effect on serum glucose or insulin, but the levels of liver enzymes for fat synthesis were increased in these rats at 100 days of age. Apparently insulin status at an early age produces long-lasting effects in the liver. ARS scientists are investigating what modifications of diet early in life influence insulin status in ways that will produce long-lasting effects on metabolism.

Dietary Sugars Regulate Cholesterol Synthesis. Further evidence of the importance of dietary carbohydrate in regulating fat and cholesterol metabolism has been obtained from research done in India under a special foreign currency grant. The research findings supplement those from other studies being made by ARS nutritionists. The diets contained about 12 percent of total calories from glucose, fructose, or sucrose in addition to cornstarch. In 1969, sugars provided about 23 percent of the total calories in U.S. diets. Even at the low levels studied, characteristic differences were seen in the effects of the sugars on fat metabolism in rats. The differences were mainly due to the stimulation of lipid synthesis in the liver coupled with a lowering of the rate of breakdown of specific lipid constituents.

Blood cholesterol was increased when sucrose was fed, reflecting an increase in liver synthesis of cholesterol. Sucrose was the only sugar to appreciably affect blood cholesterol. Dietary glucose increased cholesterol synthesis in the liver but also its breakdown; with fructose, both synthesis and breakdown were slowed.

This is the first demonstration that such low levels of glucose, fructose, and sucrose, when fed along with starch, could change the pattern of fat metabolism. Earlier work by ARS nutritionists had shown that some strains of rats were more readily affected by dietary carbohydrates than other strains. Similar differences have been seen among individuals. The findings from the Indian research, if confirmed for human subjects, may mean that some individuals need to be selective in choosing the type of sugar in their diets in order to maintain blood cholesterol in normal ranges.

Review of Human Requirements for Vitamin A. The third in a series of reviews of the world's literature on the nutrient requirements of man has been completed by ARS scientists. It is on vitamin A requirements. The review shows that in the past 40-odd years since the vitamin was discovered, research on human vitamin A requirements has been concentrated on the needs of infants and adults. There have been no reports of controlled studies with adolescents, pregnant women, or elderly people.

The review points out the need for more research on the usefulness of vitamin A from different sources, for the development of better methods of measuring vitamin A requirement, and for the extension of the research to cover the gap between infants and adults and the elderly.

Interpretation of the studies on vitamin A requirements is complicated by the fact that vitamin A may be found preformed (retinol) or as pro-vitamins (carotenoids) which may be converted into the vitamin. The usefulness of the vitamin differs according to its source. Another complication in interpreting the results is the variety of methods and standards by which requirement is measured. Dark adaptation and physical condition of the skin and of the eyes are among the measures most used. None of these criteria allows for a precise evaluation of requirements.

Vanadium may be an essential mineral. By raising chickens in a strictly controlled environment which allows the exclusion of airborne and dietary trace metal contamination, scientists in ARS have been able to produce symptoms of a vanadium deficiency. Feeding the chicks an experimental diet with a very low content of vanadium but containing all other known essential nutrients results in a significant reduction of feather growth. Vanadium has been long suspected to play an important role in metabolism but this is the first clear proof of its essential role. These results will undoubtedly stimulate much interest in the potential requirement for vanadium in human nutrition.

Body Uses Unsaturated Trans Fatty Acids Like Saturated Fatty Acids. Recent research in ARS has shown that an enzyme which activates fatty acids as the first step in their use by the body treats unsaturated trans fatty acids like saturated fatty acids. Some trans acids are formed during the hydrogenation of fats. Before any of the processes involving the use of fats by the body can occur, the fatty acids must be made into high energy compounds. These high energy compounds have been studied using rat liver fractions as a source of the enzyme, a substance which transforms the fatty acids to a high energy state. The enzyme is apparently not subject to dietary control. The enzyme activity was not affected by 48-hour fasting or by 48-hour fasting followed by 48 hours refeeding of either a normal or fat-free diet. This research adds further evidence that hydrogenated fats are used by the body in an efficient manner similar to saturated fats and have similar nutritive value.

HUMAN REQUIREMENTS FOR FATS AND FOODS TO MEET THESE NEEDS

Problems and Objectives

Cardiovascular diseases are the primary cause of death in the U.S.A., accounting for 54 percent of all deaths. Also, untold numbers of cases of morbidity and mortality in various stages of this disease afflict man causing enormous health problems. The most prevalent form of cardiovascular disease is atherosclerosis characterized by the accumulation of fatty deposits on the walls of medium and large size arteries. Fat metabolism is known to respond to changes in dietary fat. However, the desirable patterns of fat metabolism, the manner in which diet influences them, and the implications for physiological response and health are not well understood.

Among urgent problems in research on human nutrition and dietary needs, high priority is given to determining the optimal amount of fat needed in the diet and perhaps of even greater importance, the type of fat. There is ample evidence that amount and type of fat are important, and present knowledge suggests that the general recommendations for dietary fat should be lowered. Over the years there have been changes in both the amount and type of fat consumed in U.S. diets. Also, technology advances have brought about changes in fats. The changes have nutritional significance but are inadequately documented.

Nutritional requirements must be expressed in terms of foods and diets if advances in nutrition knowledge are to benefit people. The relationship of dietary fat to heart disease, atherosclerosis, blood coagulation and thrombosis has been suggested by many studies but the cause and effect hypothesis has not been proven. When nutrition research has progressed sufficiently that specific dietary recommendations can be made, it may be possible to modify a sizable percentage of the heart and vasculatory cases and increase the productive lifespan and work efficiency of people. The magnitude of the potential benefits can be appreciated if one notes that some 28 million adults in 1960-1962 were diagnosed or suspected to have heart and vasculatory disease. The economic costs of death from heart disease were calculated at nearly $32 billion annually.

Major objectives of the research are to develop recommendations for fat intake by humans and to identify the amount, kind, and assortment of foods needed. This will involve:

1. Identifying the individuals or groups of individuals who could benefit from dietary regulation of fat and the age the regulation should begin.

2. Finding out what fat constituents, such as cholesterol and fatty acids in the diet, need to be regulated and how they should be regulated.

3. Determining the amount, composition, and availability of the various lipid components in food.

4. Determining the extent and manner by which non-fat diet constituents may influence recommendations for fat intake.

A. Moderate Reduction of Blood Lipids in Man by Dietary Means

Elevated lipid patterns of blood of man are associated with cardiovascular disease, the leading cause of death in the U.S.A. The most serious attempts to reduce blood lipids have been through the substitution of diets high in polyunsaturated fatty acids for saturated fats. Decreases in blood lipids have been achieved by this means but not to a sufficient degree. We should consider therefore a moderate reduction in total fat intake from the 40-50 percent level typical of the U.S.A., to 30-35 percent. Experiments underway will test long-term diets in healthy man in various decades of life containing less fat. In this manner, we hope to predict the amount of meat, dairy products, and poultry that can be eaten to maintain blood cholesterol at 200 mg. percent or less.

B. Polyunsaturated Fatty Acids and Prostaglandins

Prostaglandins are a family of compounds with a variety of biological activities derived from the polyunsaturated essential fatty acids (EFA). Some investigators have interpreted the symptoms of EFA deficiency on the basis of impaired prostaglandin function; others have found that prostaglandins have no effect on the symptoms of EFA deficiency. Studies are now underway to elucidate the relationship of EFA status to prostaglandin function. This kind of information is needed to determine whether the nutritional status of the individual influences the prostaglandin status, which in turn would influence all the functions carried out by this class of compounds.

C. Biotin Status, Lipid Metabolism and Insulin Response in Adult Inbred Rats

We are continuing to determine the cause of one type of genetic obesity observed in a line of rats inbred from our laboratory BHE strain. The exhibition of high blood cholesterol and total lipid, or of high blood glucose or insulin, constitutes a syndrome associated with obesity in some human beings. Symptoms of biotin deficiency, also associated with abnormal glucose utilization and increased cholesterol synthesis in adipose tissue, are observed in some individual rats in the inbred line under study. Studies were conducted on adult male rats with a tendency toward high blood lipids. The rats fed a semipurified diet with an oral supplement of biotin for four weeks had higher plasma

insulin levels than unsupplemented or chow-fed littermates. In chow-fed littermates, there was a significant positive relationship between liver biotin contents and plasma insulin levels of individual animals but not between their biotin intakes and insulin levels. Biotin supplementation had no effect on mean plasma glucose levels observed in either chow-fed or sucrose-fed animals.

Effect of Biotin Supplementation on the Level of Serum Immunoreactive Insulin (IRI) in Adult Male and Female Rats

Group	Treatment	Diet	IRI (μ units/ml serum)		Glucose (mg/100 ml)	
			Males	Females	Males	Females
1[a]	None	Chow	57 ± 4[b]	44 ± 4	139 ± 10	121 ± 7
2	None	Chow	43 ± 6	54 ± 5	146 ± 9	108 ± 13
3	None	Semisynthetic	43 ± 6	52 ± 5	169 ± 8	130 ± 10
4	8 μg biotin/day	Semisynthetic	66[c] ± 7	45 ± 5	177 ± 12	128 ± 8

a Initial reference group, sacrificed at the beginning of the four-week study.
b Standard error of the mean.
c This value is significantly greater than the values for males of groups 2 and 3 ($P < .05$).

The data suggest that biotin may enhance the synthesis or release of insulin but not the conversion to its active form. Biotin tissue levels but not biotin intakes were related to insulin levels of these rats. Hopefully, evaluation of the data on the carcass and plasma lipid levels to determine their relation to biotin status of these rats, and other supplementary studies, should provide information on the role of biotin in the regulation of lipid metabolism.

D. Dietary Calcium and Lipid Metabolism

Increasing increments of dietary calcium reduces the digestibility of saturated fats. Blood lipids are decreased when dietary calcium is increased, and, conversely, blood lipids are elevated when calcium is fed at suboptimal levels. Epidemiologic studies in areas where the drinking water is hard suggest a decreased prevalence of cardiovascular disease and that calcium may be the

mineral having this effect. Studies are now underway to elucidate the regulatory role of calcium on lipid metabolism. This type of information is needed to evaluate to what degree dietary calcium plays a part in reducing the incidence of heart disease.

E. Plasma Lecithin Cholesterol Acyltransferase (LCAT)

This enzyme, which transfers fatty acids from the β-position of lecithin to cholesterol, is probably the principal source of plasma cholesteryl esters in man. Its role in cholesteryl ester formation in other species is not as yet clarified. It is well established that LCAT activity is markedly increased in essential fatty acid (EFA) deficiency. The relation of nutrition status to the activity of this enzyme has not been thoroughly investigated. Preliminary studies have shown that fasted rats maintained on a chow diet appear to have a slightly greater activity than non-fasted animals. This effect is not apparent when animals were maintained on a 16 percent corn oil diet. Studies are now underway to clarify the interrelationships of EFA status, and dietary cholesterol on the fluctuations of LCAT activity induced by fasting.

F. Lipid Structures in Processed Fats

Work on the structure and identity of the lipids in 10 margarines available to consumers included examination for gross fatty acid composition, total trans acids, mono-unsaturated and di-unsaturated cis, trans, and positional isomers, triglyceride distribution and tocopherol content. Soft margarines generally, but not always, were more unsaturated than the related standard margarine. Up to 46 percent of the monoene unsaturation, and up to 26 percent of the diene unsaturation, was trans. Calculation of P/S ratios would be more meaningful if they reflected the amounts of trans acids found in processed fats. The alpha tocopherol (vitamin E) content was found to be low relative to the amount of polyunsaturated acid present.

G. Tocopherols in Foods

The determination of individual food tocopherols has presented some difficulty. Work is underway to provide a sensitive, specific method suitable for routine use, particularly by contractors collecting food composition data. Results from the analysis of 39 selected foods by a prescribed method have been received from one of two contractors; these compare well with similar data from our own laboratory. Proper evaluation of the method and the data will be carried out following receipt of the results from a second laboratory.

Tocopherols in Vegetable Oils, mg/100 g

Oil	No. of values averaged	α-T	α-T-3	β-T	β-T-3	γ-T	γ-T-3	δ-T	δ-T-3
Coconut	1	0.5	0.5		0.1		1.9	0.6	
Corn	8	11.2		5.0[a]		60.2		1.8[b]	
Cottonseed	9	38.9				38.7			
Neem	1					58		59	
Olive	4	5.1							
Palm	4	25.6	14.3[a]		3.2[a]	31.6[b]	28.6[a]	7.0[a]	6.9[a]
Peanut	11	13.0				21.4		2.1[c]	
Rapeseed	5	18.4				38.0		1.2[a]	
Safflower	3	38.7				17.4		24[a]	
Sesame	2	13.6				29.0			
Soybean	14	10.1				59.3		26.4[d]	
Sunflower	10	48.7				5.1		0.8[b]	
Walnut	1	56.3				59.5		45.0	
Wheat germ	3	133.0	2.6[a]	71.0	18.1[b]	26.0[a]		27.1[a]	
Mustardseed	1	8.6				17.6		5.8	

a One value reported.
b Average of two reported values.
c Average of four reported values.
d Average of 12 reported values.

H. Fatty Acid Activation

An investigation of the liver enzyme system responsible for the formation of fatty acid coenzyme-A ester has shown that the structure of dietary fatty acids affects their activation. The coenzyme-A ester is an essential intermediate in the body's use of fatty acids for either energy or structure. Trans acids resemble saturated fatty acids rather than the related cis acids. Activation also varies with the position of the double bond in mono- unsaturated acids, with a minimum at the Δ^9 position. No response of activation specific to fasting or diet manipulation was found, contrary to other reports. It is postulated that there may be two enzyme systems for activating fatty acids rather than only one. This knowledge of the differences in fatty acid usage at the molecular level is basic to an understanding of how food fats differ in their dietary effects.

I. Level of Dietary Protein Affects Blood Lipids

Evidence that a high level of dietary protein increases serum cholesterol and decreases serum phospholipids was obtained in a metabolic study of six young men, 18-20 years old. They consumed diets providing either 48 or 141 grams of protein. Serum cholesterol levels were higher when the men consumed diets providing 141 grams of protein than when they ate diets containing 48 grams of protein. Levels of phospholipids in serum and linoleate in erythrocytes (red blood cells) were lower at the higher intake of dietary protein.

The protein content of the diet was altered without changing the ratio of fat to carbohydrate kilocalories, and of simple to complex carbohydrates. The fatty acid patterns of the two diets were identical, but the high protein diet containing 5 percent less fat provided slightly less linoleic acid than the low protein diet. This probably accounts for the lower linoleate in erythrocytes of subjects on the higher level of protein.

Previous studies of the effect of level of protein on blood lipids have usually been confounded by changes in the proportion of calories from fat and carbohydrate. The effect of level of dietary protein on blood lipids in humans should be further investigated. The findings reported here are part of a study of the magnesium requirements of young men conducted under an ARS research contract at the University of Wisconsin.

Publications

Benton, N. M. and Benton, D. A. 1971. Short Term Response of Serum Cholesterol to Low Cholesterol-Coconut Oil Diet Among $Le^a(+)$ and Le^a (-) men. Fed. Proc. 30: 348 (Abstract).

Berdanier, C. D., and Marshall, M. W. 1971. Biotin Intake and Insulin Response in Adult Rats. Nutr. Rpt. Int. 3(6): 383-388.

Bodwell, C. E., and Finegold, H. 1971. Conformations of Tropomyosin and Sodium Dodecyl Sulfate-Tropomyosin. Fed. Proc. 30, Part, II #315, p. 1107 (Abstract).

Elson, C. E., Humleker, E. and Pascal, L. 1971. Dietary Protein Level and Serum and Erythrocyte Lipids in Young Adult Males. Am. J. Clin. Nutr. 24: 194-199.

Lehmann, J., and Slover, H. T. 1971. Determination of Plasma Tocopherols by Gas Liquid Chromatography. Lipids 6: 35-39.

Lippel, K. 1971. Regulation of Rat Liver Acyl-CoA Synthetase Activity. Biochim. Biophys. Acta. In Press.

Lippel, K. 1971. Activation of Trans-Unsaturated Long-Chain Fatty Acids. J. Am. Oil Chem. Soc. In Press (Abstract).

Lipton, S. H., Finegold, H., and Dutky, R. C. 1971. N-(2-furacyl)Glycine - A Furan Amino Acid Derived From Fructose-Glycine. J. Agr. Food Chem. 19: 125-129.

Marshall, M. W., Durand, A. M. A., and Adams, M. 1969. Different Characteristics of Rat Strains: Lipid Metabolism and Response to Diet. In Defining the Laboratory Animal IV. Sym. of Int. Comm. on Lab. Ani. Nat. Acad. Sci., Wash., D. C. 381-413.

Morris, R. S., Frazier, W. R., Young, M., Rencz, K. H., and Benton, D. A. 1970. Effects of Long-Term Feeding of Egg on the Plasma Lipid Composition of Two Strains of Rats. Nutr. Rpt. Int. 2(3): 119-132.

Slover, H. T. 1971. Tocopherols in Foods and Fats. Lipids 6(5): 291-296.

Problems and Objectives

Although the human body contains a large variety of mineral elements, Recommended Dietary Allowances issued by the National Academy of Sciences-National Research Council have been proposed for only five minerals. Prior to 1968, only calcium and iron were included. This reflects the dearth of evidence for both minimum and maximum intake of a variety of essential mineral elements known to be required by man. Even when these requirements are known, they must be translated into terms of food and diets if advances in nutritional knowledge are to benefit people.

Benefits from mineral nutrition research may be expected to include improved health, a longer productive life, and fewer work days lost. The research leads suggest that optimal intake of minerals in the diet could lead to stronger and better developed bones and teeth with less likelihood of deterioration as in osteoporosis and tooth decay, a lower incidence of anemia with associated reduced stamina and activity, reduced incidence of goiter, a lowering of the incidence of cardiovascular disorders, and numerous other health benefits that can be translated into economic benefits.

Major objectives of the research are aimed at developing recommendations for mineral intake by humans and identification of the amount, kind, and assortment of foods needed. This includes research to:

1. Identify the individuals or groups of individuals who could benefit by altering or regulating their dietary mineral intake.

2. Identify the minerals which need to be regulated in the diet and how they should be regulated.

3. Determine the amount, composition, and availability of the mineral components of food.

Progress

A. Occurrence and Availability of Trace Elements in Foods

In part because of the accomplishments of our researches, the list of essential elements of interest to the experimental nutritionist is continuously expanding. In addition, new elements have become of practical interest in human nutrition, and the group of these is also growing.

Iron One such example is a grant for research on the availability of dietary and chemical forms of iron conducted at the University of Washington in Seattle. One of the most pressing questions asked at the Iron Workshop of the Food and Nutrition Board concerned the availability of the iron in the enriched product as consumed. The Seattle studies have shown that the baking of bread reduces the availability of ferrous sulfate ($FeSO_4$), and that in the baked product ferrous sulfate and reduced iron are about equally available. Another important finding is the validity of an extrinsic tag for many forms of iron, except heme iron. Even though this conclusion came from experiments with rats, it is most probably applicable to man. The absorption by the rat of iron from vegetable sources was increased when veal was added to the diet; this adds still another dietary influence to the complex picture of iron availability. The poor availability of egg yolk iron was significantly increased when 30 mg. of ascorbic acid were added per 100 g diet. Since this concentration is physiological, these findings suggest another function of vitamin C, in addition to those known.

Dietary Ascorbic Acid Level and Hemoglobin Response To Egg Yolk Iron 1/

Added dietary ascorbic acid, %	Number of animals	4-week gain	Hemoglobin
		g	g/100 ml
0.00	17	141±4 2/	8.4±0.3(a) 3/
0.03	18	131±5	9.6±0.2(b)
0.06	18	138±7	10.8±0.3(c)
0.125	12	144±4	12.5±0.4(d)
0.25	12	157±5	12.0±0.3(d)
0.5	13	149±6	12.9±0.4(d)
1.0	11	147±5	13.1±0.3(d)

1/ Basal yolk diet.
2/ All values mean ± standard error of the mean.
3/ Hemoglobin values with different letters differ significantly, $P < 0.01$.

Iron deficiency anemia has been found to be one of the most prevalent disorders of childhood in the United States. A project, supported by an ARS grant, is now underway at Beth Israel Hospital in Boston to assess the benefits to young children of iron-fortified milk and cereals under practical conditions.

Chromium An even greater problem of availability than with iron exists for the essential trace element chromium. None of the chemically defined chromium complexes even remotely approach the biological value of a natural chromium complex, designated glucose tolerance factor (GTF). Significant progress has been made in developing practical purification procedures from yeast that can be used on a pilot plant scale. A pilot plant operation was started and the procedures developed in the laboratory were found to work well. A number of different potential sources of GTF were assayed with the result that brewers yeast is still the best.

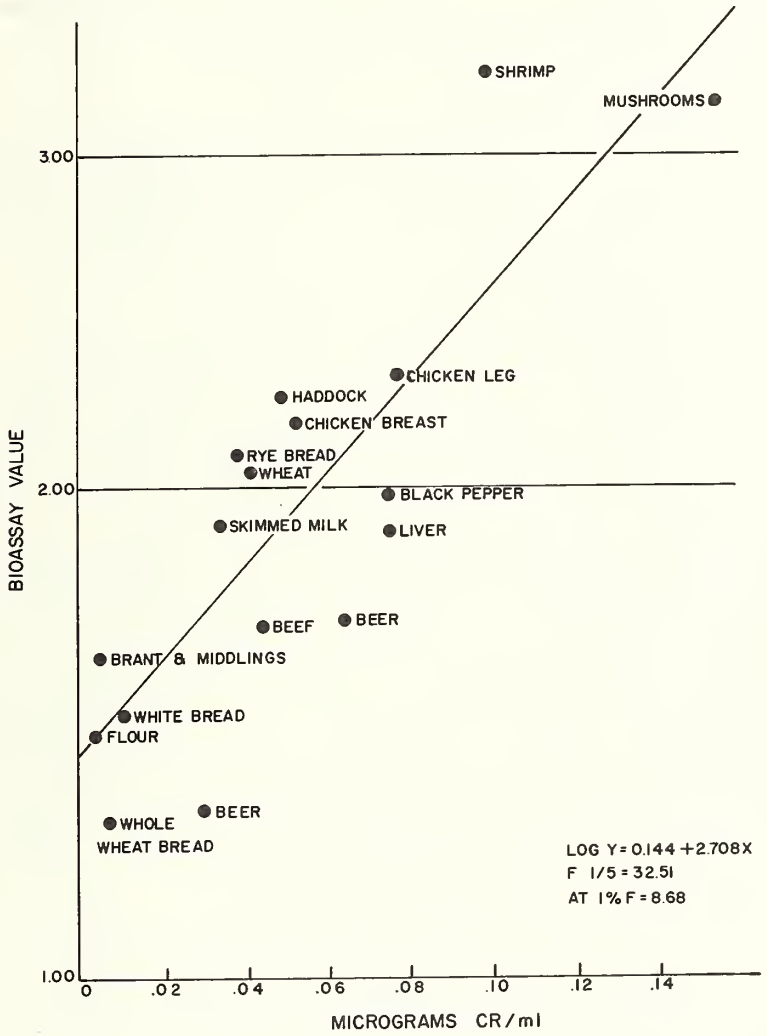
In an attempt to produce useful information on the concentration of biologically useful chromium in foods, the preliminary extraction procedure for getting GTF activity from yeast has been used with several common food items. Total activity found in acid-hydrolyzed samples and activity of the extracted material from the samples were compared to their chromium content. No significant relationship was found between total chromium and GTF activity of the acid-hydrolyzed material. The response, linearly, was relatively small. However, a highly significant relationship was found in the extracted materials and the response was four to five times greater than in the acid-treated materials. In food items such as fruits, potatoes, carrots, and eggs, little or no GTF activity was found in extracted material while extracts of whole wheat bread, meats, beer, mushrooms, and pepper showed the significant relationship. The nonspecific response to total chromium as compared to chromium present in simple extracts could be a means of evaluating foods for biologically available chromium. (See graph - next page)

Attempts to find a microbiological assay for GTF activity have produced encouraging results. Flavobacterium is inhibited by acetate, and this growth inhibition is counteracted by GTF preparations.

Although it is well established that GTF potentiates the action of insulin, the exact nature of this potentiation is unknown. Partially purified GTF, and (I^{125})-insulin were used to examine GTF binding to insulin. When the effect on glucose uptake of insulin reacted with GTF was compared with that of insulin not previously reacted with GTF, the insulin-GTF preparation produced an effect nearly five times greater than the insulin alone. Furthermore, when GTF, exclusive of insulin, was applied to an

identical column and the fraction corresponding to the elution volume of insulin was assayed, there was no effect on glucose uptake. These results demonstrate that GTF binds to insulin.

Relationship Between Chromium Content of Food Extracts and GTF Activity



Selenium Investigations concerning the selenium (Se) status of the American dietary have been continued with emphasis on the effects of cooking on the selenium content of foods. Broiling had little or no effect on the selenium content of meats. On the other hand, boiled mushrooms contained only 0.77 ppm Se even though the fresh mushrooms contained 1.44 ppm Se. Asparagus also

lost significant amounts of selenium on boiling since the fresh vegetable contained 0.96 ppm Se whereas after boiling only 0.67 ppm could be found.

Selenium Content of Meats

Product	µg Se/gram 1/
Beef	
Round steak	0.363, 0.318
Ground	0.208, 0.189
Liver	0.454, 0.409
Kidney	1.41, 1.69
Pork	
Chop	0.217, 0.261
Kidney	1.89, 1.90
Lamb	
Chop	0.172, 0.184
Kidney	1.38, 1.47
Chicken	
Breast	0.106, 0.125
Leg	0.121, 0.151
Skin	0.154, 0.146

1/ Values represent separate assays of different aliquots taken from the same sample.

Iodine Scientists at the All-India Institute of Medical Sciences in New Delhi, India completed research supported by a special foreign currency grant for the study of iodine metabolism and stores in an iodine deficient population. They produced evidence that the thyroid glands of rats reared on iodine deficient diets have different rates of iodine incorporation into iodoproteins than thyroids of rats fed a diet adequate in iodine. The pathways favor incorporation of iodine into the biologically more active triiodothyronine over that into thyroxine. This possibly is a compensatory mechanism for adapting to a low dietary iodine intake.

B. Requirement for "New" Trace Elements

A systematic effort has been made to determine nutritional requirement and biological function for trace elements for which no requirement has been established as yet but which are consistently detected in plants and animals when modern, sensitive methods are applied.

To this end, animals are raised in an all plastic, controlled environment allowing maximal exclusion of metallic contamination. The metal content of dietary components is analyzed, and experimental diets are designed that contain all presently known essential ingredients but are low in the trace element under study.

Vanadium Our attempts to define biological roles for "new" trace elements have produced strong evidence that vanadium is an essential element. After 4 weeks on a vanadium-deficient diet, chicks and Japanese quail exhibited a consistent and significant reduction of feather growth, when kept in the isolator environment. Vanadium in the diet prevented this impairment. Rats are known to have a lesser requirement for micronutrients. But even this species, bred for four generations in the low-vanadium environment, showed symptoms of a marginal deficiency: fourth generation females produced significantly fewer and smaller litters than vanadium-supplemented controls.

Nickel The studies concerned with the biological role of nickel were transferred to the new Human Nutrition Laboratory in Grand Forks, North Dakota. Chicks which were housed in all-plastic isolators and were fed a diet containing approximately 40 ppb nickel showed consistent and more severe symptoms of nickel deficiency when 100 ppm rhodium and 1 percent calcium were added to the diet. The symptoms affected were: (1) shortening and thickening of the long bones, (2) swelling of the hocks, (3) changes in shank skin color, and (4) dermatitis of the shank skin. Analyses thus far of tissues from these chicks have indicated that chicks which are nickel-deficient show decreased lipid in the vascular tissue (aorta included) and reduced yellow lipochrome pigments in the shank skin.

C. Effect of Level of Protein Intake on Calcium Requirement

High intakes of dietary protein may increase the requirements for calcium. During a metabolic study of the magnesium requirements of young men in their late adolescent period, 18-20 years of age, calcium intakes were held constant at 1.4 grams per day. Protein intake was 48 or 141 grams per day. At the higher level of dietary protein the young men excreted increasing amounts of calcium in the urine. Although they

absorbed more of the dietary calcium at the high level of protein intake, as evidenced by decreased fecal calcium, the net result of the high protein diet was a decrease in the calcium retention. In some cases the calcium balance was negative. The study was conducted under a contract with the University of Wisconsin in Madison. Work has now been initiated under an ARS grant to follow up this finding with further studies using varying levels of dietary calcium and protein.

Publications

Gutierrez, J. 1971. Yeast Growth Factor for Flavobacterium rhenanum. Bact. Proc. P142 (Abstract).

Hopkins, L. L. Jr., and Mohr, H. E. 1971. Effect of Vanadium Deficiency on Plasma Cholesterol of Chicks. Fed. Proc. 30(2): 462 (Abstract).

Johnson, N. E., Alcantara, E. N. and Linkswiler, H. 1970. Effect of Level of Protein Intake on Urinary and Fecal Calcium and Calcium Retention of Young Adult Males. J. Nutr. 100: 1425-1430.

Karmarkar, M. G., Kochupillai, N., Deo, M. G. and Ramalingaswamum, V. 1970. Adaptation of Thyroid Gland to Iodine Deficiency. Life Sci. 8: 1135-1141.

Lampbert, J. P. F., Simpson, R. E., Mohr, H. E. and Hopkins, L. L. Jr. 1970. Determination of Vanadium by Neutron Activation Analysis at Nanogram Levels to Formulate a Low-Vanadium Diet. J. Assoc. Off. Anal. Chem. 53(6): 1145-1150.

Levander, O. A., Young, M. L. and Meeks, S. A. 1970. Studies on the Binding of Selenium by Liver Homogenates from Rats Fed Diets Containing Either Casein or Casein Plus Linseed Oil Meal. Toxicol. Appl. Pharmacol. 16: 79-87.

Monsen, E. R. 1971. Iron Absorption from Intrinsically and Extrinsically Labeled Foods Fed to Normal and Iron-Deficient Rats. Fed. Proc. 30(2): 461 (Abstract).

Morris, V. C. and Levander, O. A. 1970. Selenium Content of Foods. J. Nutr. 100: 1383-1388.

Morris, E. R. and Greene, F. E. 1971. Utilization of the Iron of Egg Yolk for Hemoglobin Formation by the Growing Rat. Fed. Proc. 30(2): 461 (Abstract).

Roginski, E. E., Toepfer, E. W., Polansky, M. M. and Mertz, W. 1971. Chromium and Glucose Tolerance Factor Activity in Wheat. Fed. Proc. 30(2): 238 (Abstract).

Schwartz, R., Linz, M. D., Blakely, J. D., Walker, G. and MacKellar, I. 1971. Effect of Protein Intake on Magnesium and Calcium Retention in Adolescent Boys. Fed. Proc. 30(2): 345 (Abstract).

Zook, E. G., Greene, F. E., and Morris, E. R. 1970. Nutrient Composition of Selected Wheats and Wheat Products. VI. Distribution of Manganese, Copper, Nickel, Zinc, Magnesium, Lead, Tin, Cadmium, Chromium, and Selenium as Determined by Atomic Absorption Spectroscopy and Colorimetry. Cereal Chem. 47: 720-731.

Problems and Objectives

Daily dietary allowances for ten vitamins essential to man have been recommended by the National Academy of Sciences - National Research Council. Four of these (folacin and vitamins B_6, B_{12}, and E) were included for the first time in the last revision in 1968. The dietary allowances recommended for some vitamins are based on incomplete information for a number of age and sex groups. A number of other vitamins are known to be required, but information is too fragmentary to provide a basis for establishing recommendations. Recent information from both dietary and nutritional status surveys indicates that inadequate dietary intakes of vitamins may be found at all income levels in the U.S.

Current existing problems of vitamin nutrition in the U.S.A. are those of marginal intakes of vitamin A, folic acid, vitamin B_6 and perhaps pantothemic acid. There is now a greatly increased demand for more information on human requirements, the concentration and availability of vitamins in foods, and particularly on methodology to detect the consequences of marginal intake. In addition, the existing knowledge of the mode of action of vitamin E, with an established Recommended Dietary Allowance, is very poor and subject to much controversy.

Our current research focuses on the determination of vitamins and of their availability in various foods, on the identification of the mode of action of vitamin E, and on work with hitherto unidentified growth factors, possibly of vitamin nature. The main objectives of our research are to:

1. Identify the individuals or groups of individuals who could benefit from altering or regulating their dietary vitamin intake.

2. Identify the vitamins which need to be regulated in the diet and how they should be regulated.

3. Determine the amount, composition, and availability of the vitamin components of foods.

Progress

A. Vitamin A and Carotenes

The separation of carotene isomers from foods and the effects of food processing on vitamin A values was reported last year. However, the assignment of vitamin A values to the various carotenes rests on controversial evidence. In order to be able to assign an

availability factor to the individual compounds, a bioassay was set up which measures the potency of six different carotenes for promoting growth and liver storage of vitamin A in rats raised on a deficient diet. Some unexpected findings have already been made which, if confirmed, will cast doubt on the presently used conversion factors.

Stereoisomers of β-Carotene in Broccoli and Sweet Potatoes Before and After Cooking

Cooking time in minutes	Neo-β-Carotene B %	All-trans-β-Carotene %	Neo-β-Carotene U %
		BROCCOLI	
0	5.7	81.0	13.3
15	4.7	74.5	20.8
25	5.4	70.4	24.2
		SWEET POTATOES	
0	0.1	96.7	3.2
20	24.9	69.7	5.4
40	24.4	70.2	5.4

Review of the published information on vitamin A indicates that the state of research on vitamin A is not good. Controlled studies have been reported only for infants and adults. No reports were found of controlled studies with adolescents, pregnant women or elderly people. The methodology is not standardized and lacks precision. Reported requirements for infants varied from 25 to 200 IU/kg body weight and for adults from 1,500 to 12,000 IU/day.

Nutritionists in India are interested in the possible use of long-acting vitamin A supplements. A better knowledge of vitamin A toxicity is relevant to this problem as well as to the use of vitamin A supplements and fortification of foods in the United States. A special foreign currency grant in India is concerned with the effects of excessive doses of vitamin A on lipid metabolism in rats. Investigators reported an immediate lowering of phospholipid and total protein in livers of rats given a single large dose of vitamin A. Long-term dosages brought about a decrease in phospholipid and cholesterol in plasma and adrenal glands. The fact that even long-term administration did not affect total lipids, cholesterol and glycerides of liver may indicate a rather specific effect.

B. Availability of Folic Acid from Foods

Values of total folic acid content in foods are not sufficient to indicate the nutritional value of the food with regard to folate. Information on the availability to humans of the natural forms of folic acid in foods is needed for dietary recommendations.

Studies on six human subjects were carried out in a controlled metabolic ward at the University of California in Berkeley and supported by an ARS grant. The results allow two conclusions: (1) The availability of folacin in different foods varies greatly: that of egg yolk is very poor, that of liver and yeast intermediate, whereas the availability in lima beans approaches that of pure folic acid, and in some subjects appears to even surpass the latter. (2) Of the six subjects, two were extremely sensitive to the dietary source in their folic acid absorption, two were somewhat sensitive, whereas the rest seemed to absorb the folate equally well, regardless of dietary source. These individual differences suggest that some subjects have the conjugase activity to break down the polyglutamate forms of folic acid; others depend very much either on a dietary source of conjugase or on a readily available form of folic acid. It is the latter group of subjects who would be most vulnerable to folate deficiency. These considerations emphasize the continued need for research.

Typical results for six subjects are given below:

Food Folate Availability Expressed as Percent

	I	II	III	IV	V	VI
Egg yolk (cooked)	9	0	35	8	60	0
Liver (cooked)	31	46	21	28	54	31
Yeast	55	52	46	67	58	49
Lima beans (frozen, cooked)	76	181	43	140	79	48

Laboratory studies with humans and rats under a special foreign currency grant in Hadassah Medical School, Jerusalem, Israel showed the site of folate absorption to be in the jejunum. The folate in cow's and goat's milk is less well absorbed than crystalline pteroylglutamic acid (PGA). Rats absorbed about half the folate in liver. In field studies in which PGA and iron supplements were given to 126 anemic children the majority of the children responded positively to the therapy. Food intakes of 30 families having at least one anemic child were recorded in order to estimate folate

intake. The folate intake was found to be low. The survey data are being examined for information on food preparation.

C. Vitamin E and Selenium

The function and nutritional relationship between vitamin E and selenium remain unclear, in spite of considerable research. Our investigations were directed toward the participation of these micronutrients in membrane function. Both were found to have a role in sustaining the high K/Na gradients between the interior and exterior of liver cells, but these effects were not detected in kidney, muscle or red blood cells. As in other systems, the action of vitamin E was more pronounced than that of selenium. Even though they were mimicked by certain antioxidants, there was no correlation between cation fluxes and lipid peroxidation. No direct effects on the Na/K-activated ATPases in liver were found. Therefore, it is not clear yet how vitamin E and selenium act to preserve the K/Na gradient, but possibilities include membrane stabilization, facilitation of membrane conformational changes occurring during transport, or maintenance of energy producing reactions essential to the transport system.

D. Unidentified Growth Factors in Cereal Grains

The initial experiment in which growth stimulation was observed in BHE rats when certain cereal fractions were added to experimental diets was confirmed. Since the basal diet contained all known essential nutrients, it appears that the observed effect may be due to a yet unknown agent. No effects other than growth stimulation have been observed.

E. Vitamin C

Under a special foreign currency grant, investigators at the University of Calcutta in India are studying the influence of high cereal diets on the biosynthesis and utilization of vitamin C intake. It was shown that a cooked rice diet depressed the synthesis and tissue storage of ascorbic acid, as well as certain metabolic pathways related to vitamin C synthesis. It was suggested that an increased susceptibility toward lipid peroxidation in the liver microsomes of these rats might be responsible for the observed effects. The influence of vitamin E and of iron on these processes will be investigated.

Publications

Gupta, S., Dutta, Gupta, Chaudhurt, C. Sen, Ray, C., and Chatterjee, I. B. 1970. Enzymic Synthesis of L-Ascorbic Acid from Synthetic and Biological D-Glucuronon-1, 4-Lactone Conjugates. Anal. Biochem. 38: 46-55.

Levander, O. A. and Morris, V. C. 1970. Interactions of Methionine, Vitamin E and Antioxidants in Selenium Toxicity. J. Nutr. 100: 1111-1117.

Levander, O. A. and Morris, V. C. 1971. Biochemical Studies on the Mechanism of Action of Vitamin E and Selenium in Stimulating Rubidium (Rb) Transport in Vitamin E Deficient Rat Liver Slices. Fed. Proc. 30(2): 237 (Abstract).

Morris, V. C. and Levander, O. A. 1971. Effects of Vitamin E and Selenium on Rubidium (Rb) Transport by Rat Liver Slices. Fed. Proc. 30(2): 237 (Abstract).

Polansky, M. and Toepfer, E. W. 1971. Effect of Fumigation on Wheat in Storage. III. Vitamin B_6 Components of Wheat and Wheat Products. Cereal Chem. 48: 392-396.

Sweeney, J. P. and Marsh, A. C. 1970. Separation of Carotene Stereoisomers in Vegetables. J. Assoc. Off. Anal. Chem. 53(5): 937-940.

Sweeney, J. P. and Marsh, A. C. (In press) Effects of Selected Herbicides on Provitamin A Content of Vegetables. J. Agric. & Food Chem.

HUMAN REQUIREMENTS FOR PROTEIN AND AMINO ACIDS AND FOODS TO MEET THESE NEEDS

Problems and Objectives

Proteins and amino acids are the key components in body and tissue growth and development. There is increasing evidence in the scientific literature that a deficiency of these constituents during certain critical periods in human development can seriously and perhaps irreversibly impair not only physical development but mental development as well. Protein deficiency constitutes one of the major deficiency diseases throughout the world. Nevertheless, there are many gaps in our knowledge of human requirements for protein and amino acids. The defining of requirements for amino acids is made difficult by the complexities of interrelationships with other nutrients and among the amino acids themselves. Nutritional requirements must be expressed in terms of foods and diets before advances in nutritional knowledge can benefit people. Diet planning thus is dependent upon knowledge of the kind, amount and biological availability of proteins and their constituent amino acids in our food supply.

Major objectives of the research are to:

1. Identify the individuals or groups of individuals who could benefit from altering or regulating their dietary intake of protein and amino acids.

2. Identify the protein constituents which need to be regulated in the diet and how they should be regulated.

3. Determine the amount, composition, and biological availability of the protein and amino acid components of foods.

Progress

A. Collagen Metabolism

The rate of synthesis or degradation, and changes in physico-chemical characteristics of collagen may be correlated with changes in nutritional status and may have a fundamental effect on organ function. Investigations have been carried out to identify diet-induced changes in collagen metabolism. It was found that replacement of glucose with fructose as the dietary carbohydrate source resulted in skin collagen with a higher content of salt- and acid-soluble fractions and a significant reduction in cross-linked β-dimers. Protein-free diets resulted in greatly reduced quantities of salt-soluble tropocollagen, suggesting reduced collagen synthesis. A marked reduction in cross-linked β-components was observed in starved rats, suggesting inhibition of the cross-linking process.

Influence of Diet on Solubility of Rat Skin Collagen

Diet	% Salt Soluble	% Acid Soluble
65% Glucose (25% Casein)	3.4	11.3
90% Glucose (Protein-free)	1.2	12.2
65% Fructose (25% Casein)	5.3	15.2
90% Fructose (Protein-free)	1.9	15.0
Standard Chow (25% Protein)	6.9	23.8
Starved	4.1	18.9

Considerable effort has been made to characterize collagens from sources which are functionally and genetically different from and more important than rat skin. Cardiac collagen, for example, was found to be almost entirely intermolecularly cross-linked. It has also been observed that species differences occur in collagens from the same organ. The development of improved isolation procedures in this laboratory has made it possible to characterize collagens from organs and tissues whose functions are vital to good health and dependent on adequate nutrition.

B. Protein Structure

Procedures were developed for the isoelectric focusing of alkylated myosin subunits. Several small subunits were observed to have isoelectric points between pH 4.9 and 5.3, while 2 large subunits isoelectric focused between pH 6.4-6.5 and pH 6.7-7.2. The presence of the latter two large subunit bands suggests that large subunits of myosin may differ chemically. Infrared studies of tropomyosin complexed with sodium dodecyl sulfate suggested that a coiled-coil α-helix TM structure predominates. An infrared amide III band was detected in these complexes which may be related to lipoprotein membrane structure.

A trypsin-agarose affinity chromatography technique has been developed for purifying trypsin inhibitors. Four major chromatographically distinct trypsin inhibitor fractions have been isolated from peanuts and partially purified.

C. Growth and Survival on Rice Diets

The third generation of animals subsisting on low protein rice diets has been studied intensively under a contract with WARF Institute. Weaning weights of rats from dams fed rice diets were severely depressed. Maternal diets of rice supplemented with the limiting amino acids, lysine and threonine, resulted in larger weanlings although the very low total protein intake still caused stunting. Subsequent growth rates of these animals were much lower than the maximum which could be achieved with a high level of protein and adult body weights were correspondingly smaller. However, as these animals grew older, they maintained reasonably good health and survival did not appear to be correlated with past dietary history. In addition to mortality data, histopathological examination of survivors now underway should provide information which can be applied to nutritional problems in groups which subsist on low protein grain diets.

D. Effect of Diet on Aging

The long-term effects of a high level of protein intake and the large proportion of carbohydrate eaten as refined sugar in this country are poorly defined. Investigations are underway in which the level of dietary protein and the type of dietary carbohydrate are being varied in diets fed to rats throughout their whole lifespan. Preliminary results have shown that reproduction and lactation are poor on diets containing less than 8 percent protein. An interaction between this low level of protein and the type of carbohydrate was observed, i.e., rats receiving sucrose had a marked alopecia (loss of hair) not usually observed in rats fed cornstarch. At high levels of protein intake (45%) male rats genetically susceptible to nephrosis (BHE rats) excreted more urinary protein than others.

A related investigation was under a grant with the Harriet G. Bird Foundation. High protein diets increased the severity of disease processes in rat strains which had genetic tendencies toward the malfunctions. The disease processes particularly noted were progressive glomerulonephrosis and urinary tract obstruction. High protein diets had no ill effects in rat strains which had no inherited tendencies toward the development of these conditions.

E. Supplemental Value of Whey Products

Dried whey and whey treated by reverse osmosis were compared with other milk-derived products as supplements to corn, wheat, and rice-based diets. Preliminary data were confirmed; weight gains of rats and protein efficiency ratios of diets containing single grain supplemented with dried whey were similar to those of rats fed grains supplemented with a soy-whey product. Whey treated by reverse osmosis compared favorably with nonfat dried milk as a supplement to these grains. Development of kwashiorkor-like symptoms in rats was carried out to provide realistic models for studies of the supplementation of corn diets with these whey products.

Protein Efficiency Ratios of Rice-Based and Wheat-Based Diets Supplemented with Milk Products

Milk product added	Rice-based diet 1/		Wheat-based diet 2/	
	Percentage milk product added	Protein efficiency ratio 3/	Percentage milk product added	Protein efficiency ratio 3/
Nonfat dried milk	12.2	3.49 ± 0.08	11.5	3.03 ± 0.12
Plain dried whey	38.0	3.25 ± 0.11	35.8	2.69 ± 0.07
Dried RO whey	20.1	3.76 ± 0.16	18.9	3.31 ± 0.07

1/8.5 percent protein, 50 percent from milk products.
2/10 percent protein (60 percent from wheat, 40 percent from milk product).
3/Grams of weight gain per gram of protein eaten.

F. Availability of Food Protein

The nutritional availability of essential amino acids from various foods has been investigated. Animal feeding techniques based on minimal amino acid requirements have been developed for use in assessing changes in availability which may occur during cooking or other types of food processing. A technique for determining relative utilization of protein from breakfast cereals when eaten with milk has been devised and several commercial cereal preparations have been compared.

Research carried on under a grant with Cornell University has shown that cooking reduced the biological availability of the protein of beef round and beef liver, although no differences were found in digestibility coefficients.

G. Amino Acid Absorption

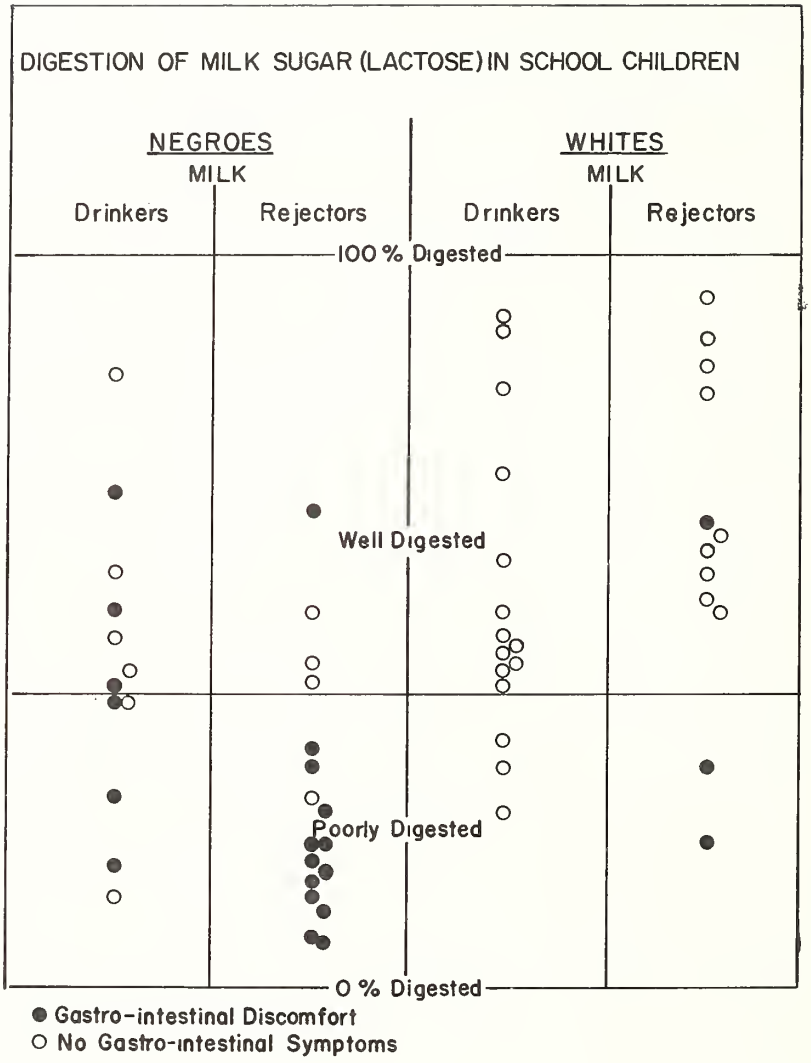
Studies were continued to investigate the effects of dietary cornstarch, fructose and sucrose on amino acid absorption by rat intestinal tissue in vitro. It was found that the uptake of several essential amino acids was greater in intestinal tissue of rats fed diets containing cornstarch than of those fed diets containing fructose and sucrose. The exception was the essential amino acid threonine; its uptake was much lower on the diets containing cornstarch. Establishing relationships between intestinal uptake of amino acids and type of dietary carbohydrate will contribute to an understanding of the effects of the presence of non-protein nutrients on the utilization of food proteins.

H. Supplementation of Rice or Ragi Diets

Under a special foreign currency grant, calculations were made of the total protein and essential amino acid contents of a variety of possible Indian diets containing various combinations of rice or ragi supplemented with indigenous legumes, green leafy vegetables, roots and tubers, and other vegetables. Chemical scores using the FAO method were computed. It was found that as many as 129 diets could be formulated which would not be limiting in lysine. However, rice diets were often limiting in sulfur-containing amino acids and ragi diets were limiting in either tryptophan or threonine. This work was done at Coimbatore, India.

I. Lactose Intolerance Among Healthy School Children

Milk rejection and lactose intolerance were compared in Negro and white children in a school lunch program in Baltimore. Twenty percent of over 300 Negro children and 10 percent of approximately 200 white children consumed less than half of their milk allotment. Lactose tolerance tests were carried out on randomly selected samples of participating children. Of the Negro non-milk drinkers selected, 76.5 percent had abnormally low responses to the lactose load test, in contrast to 17 percent of the white non-milk drinkers. Comparable figures for Negro milk drinkers were 35.7 percent and 20 percent for white milk drinkers. This work was carried out under a grant with The Johns Hopkins University.


DIGESTION OF MILK SUGAR (LACTOSE) IN SCHOOL CHILDREN
NEGROES
MILK
Drinkers
Rejectors
WHITES
MILK
Drinkers
Rejectors
100 % Digested
Well Digested
Poorly Digested
0 % Digested
● Gastro-intestinal Discomfort
○ No Gastro-intestinal Symptoms

Publications

Abernathy, R. P., Ritchey, S. J., and Gorman, J. C. 1971. Lack of Response by Preadolescent Girls to Supplementation of Low-Protein Diets with Lysine, Methionine, and Threonine. Fed. Proc. 30 (2): 586. (Abstract)

Bayless, T. M., Paige, D. M., and Ferry, G. D. 1971. Lactose Intolerance and Milk Drinking Habits. Gastroenterol. 60: 605-608.

Bechtel, P. J., Pearson, A. M., and Bodwell, C. E. 1971. Isoelectric Focusing of Myosin Subunits. Fed. Proc. 30 (3): 1310. (Abstract)

Bodwell, C. E., and Finegold, H. 1971. Conformations of Tropomyosin and Sodium Dodecyl Sulfate-Tropomyosin. Fed. Proc. 30 (3): 1107 (Abstract).

Bradfield, R. B., Jelliffe, E. F. P., and Neill, J. 1970. A Comparison of Hair Root Morphology and Arm Circumference as Field Tests of Protein-Calorie Malnutrition. J. Trop. Ped. 16: 195-196.

Bradfield, R. B. 1971. Protein Deprivation: Comparative Response of Hair Roots, Serum Protein and Urinary Nitrogen. Am. J. Clin. Nutr. 24: 405-410.

Edwards, C. H., Williamson, G. G., Craven, R., and Ganapathy, S. N. 1971. Amino Acids in Excreta of Adult Men Fed Wheat Diets. Fed. Proc. 30 (2): 299. (Abstract)

Edwards, C. H., Booker, L. K., Rumph, C. H., Wright, W. G., and Ganapathy, S. N. 1971. Utilization of Wheat by Adult Man: Nitrogen Metabolism, Plasma Amino Acids and Lipids. Am. J. Clin. Nutr. 24: 181-193.

Hseuh, A. M., and Chow, B. F. 1971. Nitrogen Balance Study of "Poor" and "Rich" Children on Fortified Wheat Products. Fed. Proc. 30 (2): 585. (Abstract)

Irwin, M. I. 1970. Current Status of Our Knowledge of Protein and Amino Acid Requirements of People. Proc. 23rd Annual Reciprocal Meat Conference of the American Meat Science Association, Gainesville, Florida, June 23-26, 186-211.

Irwin, M. I., and Hegsted, D. M. 1971. A Conspectus of Research on Protein Requirements of Man. J. Nutr. 101: 385-429.

Irwin, M. I., and Hegsted, D. M. 1971. A Conspectus of Research on Amino Acid Requirements of Man. J. Nutr. 101: 539-566.

Lipton, S. H., Finegold, H., and Dutky, R. C. 1971. N-(2-Furacyl)glycine - A Furan Amino Acid Derived from Fructose-Glycine. J. Agr. Food Chem. 19: 125-129.

McClain, P. E., Creed, G. J., Wiley, E. R., and Hornstein, I. 1970. Effect of Postmortem Aging on the Isolation of Intramuscular Connective Tissue. J. Food Sci. 35: 258-259.

McClain, P. E., Creed, G. J., Wiley, E. R., and Gerrits, R. J. 1970. Cross-Linking Characteristics of Collagen from Porcine Intramuscular Connective Tissue: Variation Between Muscles. Biochem. Biophys. Acta 221: 349-356.

McClain, P. E., Wiley, E. R., Bodwell, C. E., and Hornstein, I. 1971. Amino Acid Composition and Cross-Linking Characteristics of Collagen from the Intramuscular Connective Tissues of Striated Muscle. Int. J. Biochem. 2: 121-124.

McClain, P. E., Wiley, E. R., and McCague, K. E. 1971. Species Variation in the Cross-Linking Characteristics of Collagen from the Intramuscular Connective Tissues of Striated Muscles. Int. J. Biochem. 2: 167-172.

McClain, P. E., and Wiley, E. R. 1971. Influence of Diet on Metabolism and Cross-Linking in Rat Skin Collagen. Fed. Proc. 30 (2): 402. (Abstract)

McClain, P. E., and Wiley, E. R. 1971. Differential Scanning Calorimeter Studies of the Thermal Characteristics of Collagen. Fed. Proc. 30 (3): 1249. (Abstract)

Miles, C. W. 1970. Excretion of Hydroxyproline and Creatinine by Children Consuming Low Protein Diets. M. S. Thesis. Virginia Polytechnic Institute and State University.

Morris, S. C., and McClain, P. E. 1971. Comparative Studies of Cardiac and Skeletal Muscle Collagen. Fed. Proc. 30 (3): 1250. (Abstract)

Paige, D. M., Bayless, T. M., Ferry, G. D., and Graham, G. G. 1971. Lactose Malabsorption and Milk Rejection in Negro Children. Johns Hopkins Medical Journal 129 (3): 163-169.

Spence, N. P. 1971. Sweat Nitrogen Losses from Preadolescent Girls on Low Nitrogen Intakes. M. S. Thesis. Virginia Polytechnic Institute and State University.

Spence, N. P., Abernathy, R. P., and Ritchey, S. J. 1971. Sweat Nitrogen Losses from Preadolescent Girls on Low Nitrogen Intakes. Fed. Proc. 30 (2): 586. (Abstract)

Vaughan, D. A. 1970. Nutritional Aspects of Whey as a Food. Proc. Whey Util. Conf., 78-92.

Womack, M. 1971. Glutamine and Glutamic Acid as Sources of Supplemental Nitrogen for Adult Rats. Proc. Soc. Exp. Biol. and Med. 136: 940-941.

Young, V. R., Hussein, M. A., Murray, E., and Scrimshaw, N. S. 1971. Plasma Tryptophan Response Curve and its Relation to Tryptophan Requirements in Young Adult Men. J. Nutr. 101: 45-59.

HUMAN REQUIREMENTS FOR CARBOHYDRATES AND FOODS TO MEET THESE NEEDS

Problems and Objectives

In recent years nutritionists have moved away from the concept of the carbohydrates being essentially calorie-providers, with the different types of carbohydrates being similar in most nutritional respects. This narrow viewpoint failed to indicate any urgency for studies on the various carbohydrate components of foods. The result is that much of our information on carbohydrate content of commodities is a residual figure of carbohydrate "by difference." It is now known that many species and many strains of animals as well as individual human beings show quite different metabolic responses to different dietary carbohydrates. These studies suggest that some of the benefits of carbohydrate nutrition research may relate to heart and vasculatory diseases, which result in more deaths than any other disease. The economic costs of death from heart disease have been calculated at approximately $32 billion annually. When nutrition research has progressed sufficiently that specific dietary recommendations can be made, it probably will be possible to modify a portion of the incidence of heart and vasculatory disease and increase the productive lifespan and work efficiency of people who are metabolically sensitive to dietary carbohydrates.

Major objectives of the research are to:

1. Identify the individuals or groups of individuals who could benefit from altering or regulating their intake of dietary carbohydrate.

2. Identify the carbohydrates which need to be regulated in the diet and how they should be regulated.

3. Determine the amount, composition, and biological availability of the carbohydrate components of food.

Progress

A. Carbohydrate Effects on Human Metabolism

ARS scientists have studied the response in blood and saliva of seven young women to load doses of seven different carbohydrates. Information to date indicates the response of pyruvate and lactate levels in parotid saliva of human subjects is similar to that of blood. A follow-up study was carried out through a cooperative agreement with the University of Maryland and these same ARS scientists in an attempt to determine some of the early characteristics of

carbohydrate sensitive people. For four weeks, four subjects were fed glucose and four were fed sucrose as 85 percent of the dietary carbohydrate. The subjects were then fed the alternate diet for four weeks. Each dietary period was preceded by one week of control diet containing a variety of carbohydrates. Weekly fasting samples of blood and saliva were collected. Glucose tolerance tests were made at the end of each control period and at the end of each experimental period. Twenty-four-hour collections of urine were made prior to the glucose tolerance tests. Analyses of these samples are in progress.

B. Dietary Carbohydrates and Lipid Metabolism in Humans

Scientists at the Hebrew University in Jerusalem, Israel have studied dietary carbohydrate effects on human serum and tissue lipids and lipolytic enzymes through a special foreign currency grant. About 20 subjects have been enrolled, questionnaire and data recording forms developed, and analytical methods established. The investigators estimate that by the end of the second year of the grant they may have enough subjects to reach tentative conclusions. One ARS scientist is collaborating with the Israel investigators on this subject.

C. Short-term Response to Dietary Carbohydrate in Man

The human study carried out through a grant with the University of Alabama has been completed. After dietary fructose or sucrose, mean blood pyruvate was highest and blood glucose lowest as compared to glucose and starch. Total lactate dehydrogenase (LDH) and LDH isoenzymes were not affected by kind of carbohydrate. Men had lower blood pyruvate and glucose, but higher LDH activity than women had. Urinary nitrogen, urea, creatinine, sodium, potassium, magnesium and phosphorus were not affected by kind of carbohydrate. Uric acid excretion was higher 3 hours after the fructose or sucrose than after glucose or starch. Magnesium excretion seemed to be influenced by ammonia nitrogen excretion. Women excreted less nitrogen and magnesium than men. There was no significant difference between men and women in excretion of sodium, potassium or phosphorus.

Mean Blood Pyruvic Acid Content (mg/100 ml)
for Eight Subjects After Test Meals

	Mean blood pyruvic acid, mg/100 ml									
	Men					Women				
	G	F	S	C	W	G	F	S	C	W
Fasting sample	1.04	1.02	1.10	1.09	1.13	0.89	0.89	1.00	1.02	1.03
Hours after test meal										
1	1.38	2.22	1.99	1.23	1.26	1.66	2.54	2.42	1.51	1.41
2	0.82	1.32	0.89	1.03	0.89	0.96	1.64	1.27	1.35	1.22
3	0.81	0.76	0.81	0.80	0.82	0.75	0.79	0.79	0.75	0.80
4	0.78	0.82	0.80	0.86	0.76	0.81	0.74	0.84	0.76	0.73
5	0.79	0.90	0.84	0.94	0.85	0.94	0.84	0.83	0.86	0.82

G=glucose; F=fructose; S=sucrose; C=cornstarch; W=wheatstarch.

The data showing no significant differences in lactate dehydrogenase activity in blood after the various test meals indicate that the significant differences in LDH activity found in a previous study in our laboratory after feeding starch or sucrose for 30 days were not immediate responses. They probably developed over the 30-day period and may not have reached their greatest value. These results indicate that more frequent samplings should be taken and methodology should be developed so that enzyme activity levels can be measured frequently with little trauma to the subject.

D. Dietary Carbohydrates Cause Vascular Lesions

Investigators working under a special foreign currency grant at Hadassah Medical School in Jerusalem, Israel have developed and partially defined a model system for studying the effects of dietary sucrose on degenerative disease. Their high-carbohydrate diet and their strain of rat are such that feeding sucrose in place of starch resulted in lesions of the eyes and kidneys after 8-16 months of treatment. Eye lesions included a loss in dark adaptation as well as the development of microaneurysms in the retinal capillaries. The kidneys of the sucrose-fed animals became enlarged with characteristic signs of diffuse glomerulosclerosis. These animals did not appear to be diabetic but, when fed sucrose over a long period, displayed a definite impairment of glucose tolerance. The abnormalities appeared to be caused by the vascular lesions.

Amplitude Values of B-wave From Electroretinograms of Rats Fed Sucrose and Starch Showing a Lower Sensitivity in Sucrose-fed Rats with Equal Rates of Dark Adaptation

Time in dark (min)	Sucrose-fed rats (μV)[1/]	Starch-fed rats (μV)[2/]	Multiplication Factor
0	70	102	1.46
10	135	220	1.62
20	160	270	1.69
30	177	293	1.66
40	190	310	1.64
50	200	328	1.64
60	212	338	1.59
		Total	1.61

1/Average of 8 animals.
2/Average of 7 animals.

E. Effects of Exercise Upon Carbohydrate Requirements

Scientists working under a cooperative agreement with the University of Maryland have studied effects of age and dietary carbohydrate source on the responses of rats to moderate forced exercise. Rats of two ages (weanling vs. 150 days) were fed a nutritionally adequate, moderately high-fat diet containing either cornstarch or a mixture of simple and complex carbohydrates. Under sedentary conditions, consumption of the cornstarch in place of the mixture favored the accumulation of body weight and of carcass fat. However, under conditions of forced exercise, the effect of carbohydrate source was reversed. Preliminary data indicate that adrenalectomy per se did not affect the metabolic response to exercise. The inference of this research is that dietary recommendations regarding kind as well as amount of carbohydrate will have to take into account the level of physical activity of the individual.

F. Effect of Carbohydrates Upon Insulin Levels

ARS scientists conducting research with laboratory animals have been defining, among rat strains, metabolic characteristics which influence the abnormal responses to certain dietary carbohydrates. The BHE strain and an inbred substrain have been found to have abnormally high levels of circulating insulin early in life and reduced adipose tissue sensitivity. Even after the serum insulin decreases to levels typical of other strains, these animals showed

high levels of key enzymes involved in fatty acid synthesis. This and additional evidence indicate that insulin status early in life has a long-lasting influence on the metabolic pattern of the individual at maturity. The tendency of these rats to have extra fat in the liver and carcass is specific to conditions of ad libitum feeding; liver and carcass fat of the BHE and Wistar strains were identical when animals received the same restricted food intake under conditions of meal feeding.

Effect of Age and Strain on the Fasting Level of Blood Glucose and Serum Immunoreactive Insulin in Rats

Strain	Average body weight g	Immunoreactive insulin μunits/ml serum	Whole blood glucose mg/100 ml
50 days of age			
Wistar	140 (12)[1]	15 ± 1[2,3]	83 ± 2[3]
BHE	173 (10)	99 ± 7[3]	90 ± 4
IN-BHE	175 (10)	154 ± 9[3]	94 ± 5
100 days of age			
Wistar	308 (12)	23 ± 3[3]	85 ± 2
BHE	325 (12)	42 ± 5[3]	90 ± 8
IN-BHE	368 (9)	31 ± 6[3]	88 ± 8
300 days of age			
Wistar	494 (10)	39 ± 2	108 ± 7
BHE	490 (12)	45 ± 3	100 ± 8
IN-BHE	506 (12)	26 ± 3[3]	140 ± 15[3]

1/Number of animals.
2/Standard error of the mean.
3/This value is significantly different from those of the other two groups at this age ($P < .05$) as tested by Duncan's multiple range test.

G. Carbohydrates and Enzymes for Fat Synthesis

Work has continued on the control of liver enzymes in fat synthesis, particularly during the refeeding of test diets to starved animals or the feeding of sucrose ad libitum. The effects of the kind and level of carbohydrate in the refed diets and the role of the insulin

response have been systematically studied. The inclusion of phospholipids in the diet has been found to inhibit the enzyme elevation and to lower liver lipids. Evidence has been obtained that even small amounts of dietary carbohydrate inhibit the degree that absorbed fat yields free fatty acids in plasma.

Publications

Behall, K. M., Small, M. R., Holden, J. M., and Kelsay, J. L. 1971. Parotid Saliva Amylase and Protein Secretion in Response to Different Carbohydrate Load Doses. Fed. Proc. 30: 405 (Abstract).

Berdanier, C. D., Moser, P. B., and Trout, D. L. 1971. Insulin Status of the Carbohydrate Sensitive BHE Rat. Fed. Proc. 30: 579 (Abstract).

Berdanier, C. D., Marshall, M. W., and Moser, P. 1971. Age Changes in the Level of Serum Immunoreactive Insulin in Three Strains of Rats. Life Sci. 10: 105-109.

Berdanier, C. D., Szepesi, B., Moser, P., and Diachenko, S. 1971. Insulin and Enzyme Responses of Three Strains of Rats. Proc. Soc. Exp. Biol. Med. 137: 668-673.

Berdanier, C. D., Szepesi, B., Diachenko, S., and Moser, P. 1971. Effect of Tolbutamide and Exogenous Insulin on the Metabolic Responses of Rats. Proc. Soc. Expt. Biol. Med. 137: 861.

Chang, M. L. W., and Johnson, M. A. 1971. Effect of Dietary Ingredients on Dehydrogenases of HMP Shunt and Liver Lipids of Rats. Fed. Proc. 30: 232 (Abstract).

Chang, M. L. W., Lee, J. A., and Trout, D. L. 1970. Effect of Dietary Egg and Carbohydrate on HMP Shunt Dehydrogenases and Lipids of Liver in Rats. J. Nutr. 100: 1317-1322.

Chang, M. L. W., Lee, J. A., Schuster, E. M., and Trout, D. L. 1971. Metabolic Adaptation to Dietary Carbohydrates in Two Strains of Rats at Three Ages. J. Nutr. 101: 323-329.

Cohen, A. M., Teitlebaum, A., and Bohen, B. 1970. Effect of Glucose, Fructose, Sucrose and Starch on Nitrogen Utilization in Rats Fed High and Low Protein Diets. Israel J. Med. Sci. 6: 86-90.

Cohen, A. M., Freund, H., and Auerbach, E. 1970. The Electroretinogram in Sucrose and Starch Fed Rats. Metabolism 19: 1064-1067.

Cohen, A. M., and Rosenman, A. 1971. Diffuse Glomerulosclerosis in Sucrose Fed Rats. Diabetologica 7: 25-28.

Eheart, J. F. and Mason, B. S. 1970. Nutrient Composition of Selected Wheats and Wheat Products, V. Carbohydrates. Cereal Chem. 47: 715-719.

Kelsay, J. L., Behall, K. M., Holden, J. M., and Crutchfield, H. C. 1971. Lactate and Pyruvate Levels in Blood and Parotid Saliva in Response to Load Doses of Different Carbohydrates. Fed. Proc. 30: 405 (Abstract).

Meyer, F. L., Mattox, H., Bolick, M., and MacDonald, C. 1971. Metabolic Changes after Test Meals with Different Carbohydrates: Blood Levels of Pyruvic Acid, Glucose, and Lactic Dehydrogenase. Am. J. Clin. Nutr. 24: (6): 615-621.

Szepesi, B., Berdanier, C. D., Diachenko, S. K., and Moser, P. B. 1971. Effect of Repeated Starvation on Serum Insulin Level and the Enzyme Overshoot in Liver. Can. J. Biochem. 49: 393-395.

Szepesi, B., Berdanier, C. D., and Egawa, M. 1971. Evidence for Increased Constitutiveness of NADP-linked Dehydrogenases. J. Nutr. 101: (7): 863-868.

Szepesi, B., and Moser, P. 1971. Persistence of Increased Inducibility of NADP-linked Dehydrogenases in Rat Liver. Proc. Soc. Exp. Biol. Med. 136: 200-202.

Szepesi, B., and Michaelis, O. E. IV 1971. On the Nature of the Fructose Effect on NADP-linked Dehydrogenases in Rat Liver. Fed. Proc. 30: 232 (Abstract).

Trout, D. L., and Conway, E. S. 1971. Acute vs Chronic Rates of Fat Absorption. Fed. Proc. 30: 236 (Abstract).

EFFECT OF PESTICIDE USE ON HUMAN DIETARY REQUIREMENTS

Problems and Objectives

Pesticides are used at some stage of the production of almost every food eaten by the consumer. The residue levels in human diets are below those allowed by Federal regulation except in exceptional cases. The present ban on the use of persistent types of pesticides on certain classes of food crops further reduces the likelihood of the diet being an appreciable source of pesticide chemicals. Accordingly, no new research is being initiated on the effects of intake of low levels of pesticides over long periods of time upon the metabolism of nutrients in humans. In line with present agricultural management practices, emphasis has been placed on the effect of the pesticide use during plant growth upon the nutrient content and value of foods. Progress on studies originated earlier on the relationship of pesticide residues to nutritional response also is reported.

Major objectives of the research are listed below. Ongoing research deals with objective 3. Findings also are reported for contract research funded in previous years.

1. Determine the effects of long-term, low level intakes of pesticides upon nutrient requirements of people.

2. Determine whether proper diet may protect against adverse effects of environmental levels of pesticides in the diet.

3. Identify and measure the effects of the use of pesticides during production upon the nutritive value of foods.

4. Determine whether and how recommendations for diet and use of foods need to be adjusted as the result of the use of pesticides under allowed procedures.

Progress

A. Pesticides and Nutrient Content of Foods

The carotene content of vegetables seems to be susceptible to influence by the use of pesticides during production. Carotene content was significantly increased in Danvers and Chantenay carrots grown in soil treated with linuron or CIPC. Butternut squash grown in soil treated with amiben or dinoseb also were significantly higher in carotene content than were control samples. The carotene content is being further characterized according to the stereoisomers present. Biological assays are being made for the vitamin A value of the vegetables and the effects of blanching, frozen storage, cooking, and canning of the vegetables.

Contract research with the University of Utah showed that soil fumigation with Telone at the rates of 10, 20, and 30 gal/A and Nemagon at the rates of 1, 2, and 3 gal/A one week before planting carrot and sweet corn seeds significantly increased contents of total carotenes (16-45%), beta carotene (15-48%), and total sugars (6-33%) in carrots, and total carotenoids (up to 33%) in sweet corn seeds. Diet calculations for vitamin A based on present tables of food composition may considerably underestimate the vitamin A value of vegetables grown with today's methodology.

The protein content of sweet corn and peas was increased and the amino acid pattern altered as a result of soil treatment with the s-triazines including simazine, propazine, igran, and ametryne at low concentrations. The protein content of peas were increased up to 30 percent and of corn up to 9.4 percent. Individual amino acid values were generally increased in the treated plants except for decreased levels of isoleucine, histidine, and cystine in treated peas and glutamic acid in treated corn.

B. Response to Chlorinated Hydrocarbon Insecticides when Dietary Protein is From Rice

Third generation studies have been completed of rats fed rice-based diets. The findings support those reported last year for weanlings of second generation mothers. Weanling weights of rats receiving casein were much higher than of those receiving the rice diets, even when supplemented with lysine and threonine. Growth rates followed the same pattern. Lysine and lysine plus threonine supplementation improved growth rates somewhat. Addition of DDT and malathion had no effect on weanling weights or growth rates in any of the dietary groups. These results are from contract research with the Wisconsin Alumni Research Foundation at Madison.

C. The Effect of Exercise and Diet Composition on DDT Retention and Nutrient Metabolism

A study has been conducted to determine the effect of exercise on the nutritional responses of rats fed either a high fat or high carbohydrate diet containing low levels of DDT. Weight gain, serum and liver cholesterol levels, serum insulin levels and total liver and carcass fat and protein, as well as concentrations of DDT in selected tissues have been determined. The results are being evaluated and prepared for publication. This research was conducted under a contract with the Wisconsin Alumni Research Foundation in Madison.

Publications

Matthews, R. H., Fifield, C. C., Hartsing, T. F., Storey, C. L., and Densis, N. M. 1970. Effect of Fumigation on Wheat in Storage. I. Physical Measurements of Flour. Cereal Chem. 47: 579-586.

Matthews, R. H., Fifield, C. C., and Hartsing, T. F. 1970. Effect of Fumigation of Wheat in Storage. II. Physical and Eating Quality of Bread and Rolls. Cereal Chem. 47: 587-591.

Singh, B., and Salunkhe, D. K. 1970. Some Metabolic Responses of Bush Bean Plants to a Subherbicidal Concentration of s-Triazine Compounds. Canad. J. Bot. 48: 2213.

Sweeney, J. P., and Marsh, A. C. 1970. Separation of Carotene Stereoisomers in Vegetables. J. Assoc. Off. Anal. Chem. 53(5): 937-940.

Wu, M., Singh, B., Wu, M. T., Salunkhe, D. K., and Dull, G. G. 1970. Effects of Certain Soil Fumigants on Essential Nutritive Components and the Respiratory Rate of Carrot (Daucus Carota L.) Roots. Hort. Science 5: 221.